Hepatic Malonyl-CoA Synthesis Restrains Gluconeogenesis by Suppressing Fat Oxidation, Pyruvate Carboxylation, and Amino Acid Availability

Ella Colistro
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Introduction

Liver Metabolism

- The liver alternates between de novo lipogenesis (DNL), glycogen synthesis, and protein synthesis during feeding; and fat oxidation, gluconeogenesis (GNG), and proteolysis during fasting.
- Hormones (insulin and glucagon) regulate these opposing pathways, along with metabolite-mediated controls.

Acetyl-CoA and Malonyl-CoA

- Acetyl-CoA, derived from β-oxidation, is a precursor for DNL.
- Conversion of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase (ACC) prevents the futile activation of these opposing pathways.
- ACC1 (cytosolic) supports DNL and fatty acid (FA) elongation, while ACC2 (mitochondrial) inhibits CPT-1, controlling fat oxidation.
- Malonyl-CoA modulates metabolic pathways by regulating fat oxidation, GNG, and energy production.
- Loss of ACC1/2 decreases malonyl-CoA, activates fat oxidation, and lowers liver triglycerides.

ACC Regulation

- During fasting, low insulin, high glucagon, and AMP-activated protein kinase (AMPK) inhibit ACC, reducing DNL and activating fat oxidation.
- Activation of β-oxidation increases mitochondrial acetyl-CoA, which activates pyruvate carboxylase (PC), stimulating GNG.

Ethical Considerations

- All procedures were performed in accordance with National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee at UT Southwestern Medical Center.

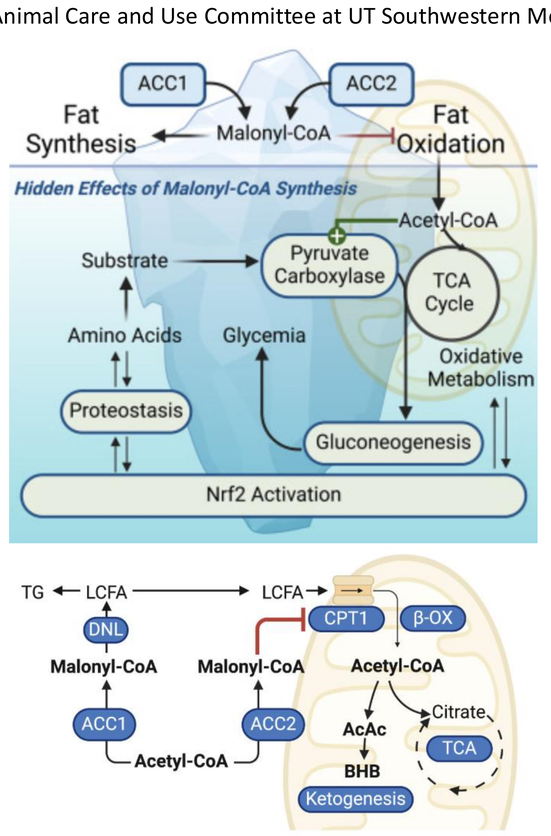


Figure 1. Schemes illustrating the role of malonyl-CoA in liver metabolism.

Aims

- Examine the role of PC in FA-induced hepatic GNG.
- Evaluate metabolic changes in LDKO mice.
- Test the necessity of CPT-1-mediated LCFA oxidation for GNG.
- Assess the effects of ACC1/2 deletion.

Hypotheses

- Loss of ACC1/2 results in increased FA oxidation.
- Increased FA oxidation stimulates hepatic GNG by activating PC, which enhances ATP production, suppressing glycolysis.
- CPT-1-mediated long-chain fatty acid (LCFA) oxidation is necessary for the increased GNG observed in LDKO mice.

Methods

Genetically Modified Mice

- Liver-specific double knockout (LDKO) mice, which lack ACC1/2, were generated by crossing mice with floxed ACC1/2 alleles with mice expressing Cre recombinase under liver-specific albumin promoter.
- This genetic modification resulted in a loss of ACC1/2 specifically in the liver, allowing the study of their role in hepatic metabolism and GNG.

In Vivo Metabolic Flux Measurements

- To investigate the role of FA oxidation in hepatic GNG, PC flux and TCA cycle turnover were measured in vivo using stable isotope tracers.
- Mice were intraperitoneally infused with anaplerotic tracers [U-$^{13}C_3$]propionate and [U-$^{13}C_3$]lactate, which generate ^{13}C-labeled isotopomers in TCA cycle intermediates.
- Plasma glucose levels were then analyzed to quantify TCA cycle metabolism and GNG intermediate incorporation.

TCA Cycle Intermediate Quantification

- To evaluate the impact of ACC1/2 deletion on hepatic TCA cycle metabolism, hepatic metabolite levels were quantified in LDKO and wild-type (WT) mice.
- TCA cycle intermediates were measured following pharmacological inhibition of ACC using MK-4074.
- The role of CPT-1-mediated LCFA oxidation was assessed by inhibiting CPT-1 with etomoxir and by genetically deleting of hepatic PC, both of which were expected to prevent the increase in TCA cycle intermediates associated with ACC inhibition.
- TCA cycle intermediates were quantified via mass spectrometry, and the results were compared across treatment groups.

Results

Enhanced PC Flux and TCA Cycle Turnover in LDKO Mice

- LDKO mice exhibited elevated PC flux and TCA cycle turnover compared to control mice.
- Infusion with anaplerotic tracers resulted in a greater incorporation of ^{13}C-labeled isotopomers into TCA cycle intermediates in LDKO mice, indicating enhanced oxidative metabolism of these metabolites (Figure 2B).
- This suggests that the loss of ACC1/2 accelerates TCA cycle flux, potentially through an increased supply of acetyl-CoA derived from FA oxidation.

Elevated Hepatic Acetyl-CoA and Citrate in LDKO Mice

- The loss of ACC1/2 in LDKO mice resulted in significantly higher levels of acetyl-CoA and citrate.
- Acetyl-CoA levels were notably higher in LDKO mice, which likely contributes to the activation of PC, a key enzyme in GNG (Figure 2D).
- Similarly, citrate levels were elevated, which is known to inhibit phosphofructokinase (PFK), further promoting GNG (Figure 2E).
- These findings were consistent with the observed increase in GNG activity in LDKO mice, as assessed by plasma glucose measurements.

TCA Cycle Intermediate Elevation in LDKO Mice

- TCA cycle intermediates were significantly elevated in the livers of LDKO mice compared to control mice (Figure 2N).
- This elevation was consistent with the increased turnover observed in the isotope tracer experiments, suggesting that ACC1/2 deletion leads to enhanced oxidative metabolism in the liver.
- To further validate this, WT mice were treated with MK-4074, resulting in similar increases in TCA cycle intermediates (Figure 2O).
- This dose-dependent effect indicates that ACC inhibition can replicate the metabolic changes seen in LDKO mice.

CPT-1-Mediated FA Oxidation Promotes TCA Cycle Activity

- The increase in TCA cycle intermediates following ACC inhibition was abolished when CPT-1 was inhibited using etomoxir or when hepatic PC was genetically deleted (Figure 2O and 2P).
- These results show that CPT-1-mediated FA oxidation plays a critical role in activating the TCA cycle and promoting GNG in the absence of ACC1/2.

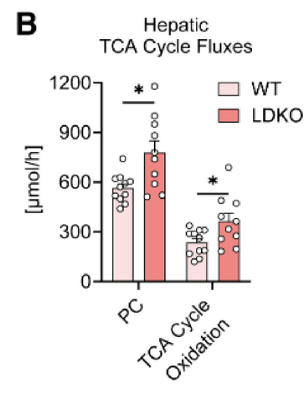


Figure 2B. *In vivo* hepatic pyruvate carboxylase (PC)-mediated anaplerosis and TCA cycle turnover estimated by $^2H/^{13}C$ MFA (n = 10–11).

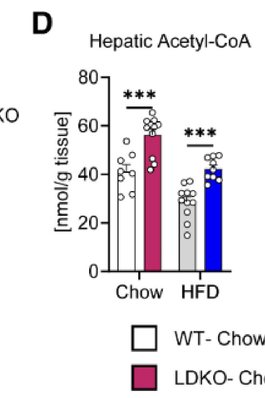


Figure 2D. Hepatic acetyl-CoA concentration in fed ice on chow and HFDs (n = 8-11).

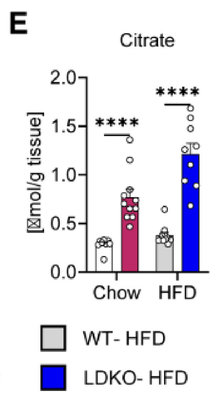
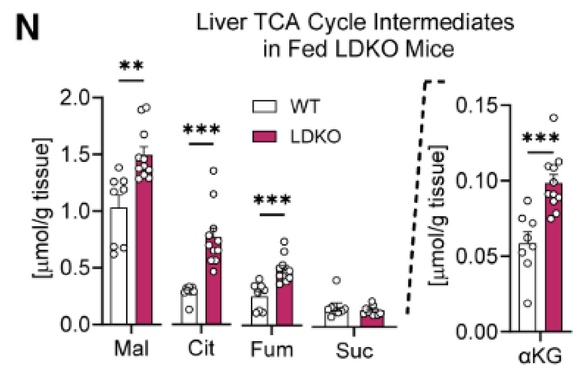
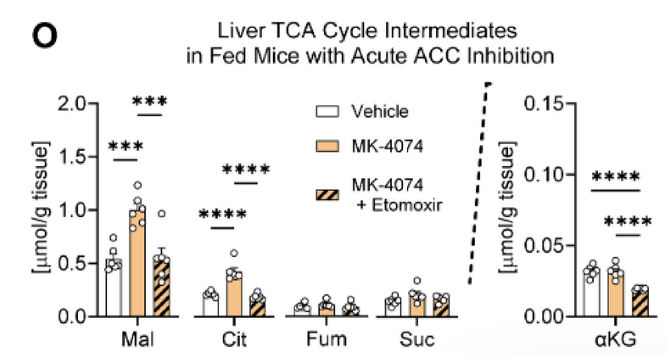


Figure 2E. Hepatic citrate concentration in fed mice on show and HFDs (n = 8-11).





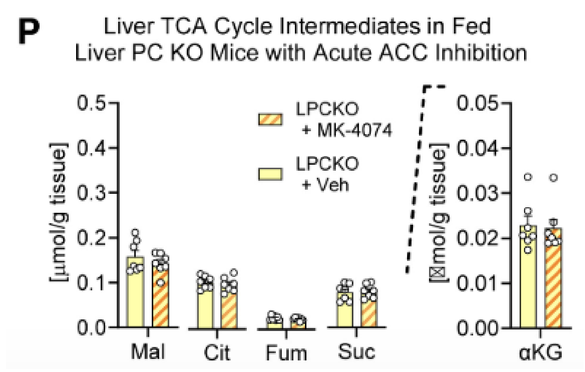


Figure 2N-O. Hepatic TCA cycle intermediate concentrations in fed (N) LDKO mice (n = 8-11), (O) C57BL/6 mice 2 h after ACC1/2 inhibition (100 mg/kg MK-4074) and CPT-1 inhibition (20 mg/kg etomoxir) (n = 6), and (P) liver-specific pyruvate carboxylase knockout mice 2 h after ACC1/2 inhibition (MK-4074 at 100 mg/kg) (n = 7).

Conclusion

These results demonstrate that the loss of ACC1/2 in LDKO mice leads to enhanced TCA cycle turnover and increased incorporation of isotopic tracers, which is accompanied by elevated hepatic TCA cycle intermediates. The data supports the hypothesis that ACC1/2 deficiency drives increased FA oxidation, which subsequently activates the TCA cycle and GNG.

Deja, S., Fletcher, J. A., Kim, C.-W., Kucejova, B., Fu, X., Mizerska, M., Villegas, M., Pudelko-Malik, N., Browder, N., Inigo-Vollmer, M., Menezes, C. J., Mishra, P., Berglund, E. D., Browning, J. D., Thyfault, J. P., Young, J. D., Horton, J. D., & Burgess, S. C. (2024). Hepatic malonyl-COA synthesis restrains gluconeogenesis by suppressing fat oxidation, pyruvate carboxylation, and amino acid availability. *Cell Metabolism*, *36*(5). https://doi.org/10.1016/j.cmet.2024.02.004